Nitrogen-Corrected Apparent Metabolizable Energy Value of Crude Glycerol for Laying Hens[1]

P. J. Lammers,* B. J. Kerr,† M. S. Honeyman,* K. Stalder,* W. A. Dozier III,‡ T. E. Weber,† M. T. Kidd,§ and K. Bregendahl*[2]

*Department of Animal Science, Iowa State University, Ames 50011; †USDA-ARS, Swine Odor and Manure Management Research Unit, Ames, IA 50011; ‡USDA-ARS Poultry Research Unit, Mississippi State, MS 39762; and §Department of Poultry Science, Mississippi State University, Mississippi State 39762

ABSTRACT An experiment was conducted with laying hens to determine the AME_n value of crude glycerol, a coproduct of biodiesel production. Crude glycerol (87% glycerol, 9% water, 0.03% methanol, 1.26% Na, and 3,625 kcal/kg of gross energy) was obtained from a commercial biodiesel production facility (Ag Processing Inc., Sergeant Bluff, IA). A total of forty-eight 40-wk-old laying hens (Hy-Line W-36) were placed in metabolic cages (2 hens/cage) and given free access to the experimental diets. A corn and soybean meal-based basal diet (18% CP, 2,875 kcal/kg of AME_n, 4.51% Ca, 0.51% nonphytate P) was formulated with 15% glucose·H_2O and 1% Celite. Four dietary treatments were created by substituting 0, 5, 10, or 15% crude glycerol for glucose·H_2O (3,640 kcal/kg of AME_n). After 7 d of dietary adaptation, excreta were collected twice daily for 3 d, freeze-dried, and analyzed for contents of DM, Kjeldahl N, acid-insoluble ash, and gross energy. Egg production was recorded daily, and eggs were collected on d 7 and 8 of the experiment for calculation of egg mass (egg production × egg weight). Feed consumption was measured over the 10-d experimental period. Egg-production data were analyzed by ANOVA with 4 treatments and 6 replications in a completely randomized experimental design. The AME_n value of crude glycerol was estimated as the slope of the linear relationship between the inclusion rate of dietary crude glycerol and the glucose-corrected AME_n value of the experimental diets. No significant treatment effects ($P > 0.1$) were apparent for egg-production rate (93.0%), egg weight (56.1 g), egg mass (52.2 g/d), or feed consumption (104 g/d). Linear regression analysis ($P < 0.001$, $r^2 = 0.92$, n = 24) revealed that the AME_n value of the crude glycerol used in this study was 3,805 ± 238 kcal/kg (mean ± SEM; as-is basis) for laying hens.

Key words: crude glycerol, biodiesel co-product, nitrogen-corrected apparent metabolizable energy, laying hen

2008 Poultry Science 87:104–107
doi:10.3382/ps.2007-00255

INTRODUCTION

In the United States, the production of diesel fuel from vegetable oil has increased exponentially from less than 2 million liters in 1999 to almost 1 billion liters in 2005 (National Biodiesel Board, 2007). Biodiesel is produced through a NaOH- or KOH-catalyzed transesterification of the triacylglycerols in oils or fats with an alcohol, usually methanol (Ma and Hanna, 1999; van Gerpen, 2005). Through this reaction, the fatty acids are methylated to form methyl alkyl esters (i.e., biodiesel), and the principal coproduct from the process is crude glycerol (Ma and Hanna, 1999; van Gerpen, 2005; Thompson and He, 2006). Using current refinement techniques, production of 1,000 L of biodiesel results in the generation of 79 kg of crude glycerol (Thompson and He, 2006). Although chemically pure glycerol is a valuable industrial compound for use in consumer products, such as cosmetics and pharmaceuticals (Ma and Hanna, 1999; Thompson and He, 2006), purification to meet United States Pharmacopeia specifications may become uneconomical given the existing capacity and projected growth in biodiesel production. Thus, substantial amounts of crude glycerol may become available for use as livestock feed at relatively economical costs.

Free glycerol only occurs in minute amounts, if at all, in feed ingredients, but glycerol is regularly consumed as part of triacylglycerols. Glycerol is a precursor to glyceraldehyde 3-phosphate, an intermediate in the lipogenesis and gluconeogenesis pathways, and yields energy through the glycolytic and tricarboxylic-acid pathways (Lin, 1977; Tao et al., 1983; Brisson et al., 2001). Studies

Received June 18, 2007.
Accepted October 4, 2007.
[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA or Iowa State University and does not imply approval to the exclusion of other products that may be suitable.
[2]Corresponding author: kristjan@iastate.edu

examining the effects of feeding chemically pure or crude glycerol from biodiesel production to broiler chickens (Simon et al., 1996; Cerrate et al., 2006), turkey hens (Rosebrough et al., 1980), and pigs (Kijora et al., 1995) have shown that glycerol can be used a source of dietary energy for livestock. There are no reports, however, in which the AME_n of crude glycerol has been directly determined. The objective of the study was to determine the AME_n of crude glycerol when fed to laying hens.

MATERIALS AND METHODS

A total of forty-eight 40-wk-old Single-Comb White Leghorn laying hens (Hy-Line W-36) were obtained from a commercial source and placed in metabolic cages (30.5 × 50.8 × 40.6 cm, width × depth × height, respectively), equipped for collection of excreta, in a light-controlled, fan-ventilated room at the Iowa State University Poultry Science Research Center. Each cage contained 2 hens and was equipped with a steel self-feeder and a trough waterer. Upon arrival, hens were given free access to a laying hen diet (17% CP, 2,875 kcal/kg of AME_n, 4.40% Ca, 0.43% nonphytate P) and water for a 2-wk acclimation period. Hens were provided with 16 h of light and 8 h of darkness per day, and the ambient temperature was maintained at 26°C throughout the study. After the 2-wk acclimatization period, hens were weighed and randomly assigned to cages. Each cage was randomly assigned to 1 of the 4 experimental diets. Hens were given free access to the experimental diets for a 7-d adaptation period followed by a 3-d collection period. All procedures relating to the use of live animals were approved by the Iowa State University Institutional Animal Care and Use Committee.

A total of 4 experimental diets were used, formulated from a basal diet (Table 1) in which 5, 10, or 15% crude glycerol (Ag Processing Inc., Sergeant Bluff, IA; Table 2) was substituted for glucose·H_2O on an equal-weight basis (Sell et al., 2001). All diets were formulated to meet or exceed the NRC (1994) nutrient recommendations and contained 1.0% Celite to increase the content of acid-insoluble ash (an indigestible marker). The AME_n contents of the experimental diets were not equalized. The basal diet was mixed in one large batch in a horizontal ribbon mixer without glucose·H_2O or crude glycerol additions; the treatment diets were subsequently prepared by mixing a portion of the basal diet with the specified amounts of glucose·H_2O and crude glycerol in a Hobart mixer (model H-600; Hobart, Troy, OH). The diets were fed in mash form.

Egg production was recorded daily and the feed consumption was determined for the 10-d experiment. Eggs collected over a 48-h period on d 7 and 8 of the experiment were weighed, and egg mass was calculated as egg production × egg weight. After 1 wk of adaptation to the experimental diets, excreta was collected twice daily for 3 d and stored at –20°C until analysis.

Excreta samples were pooled within cage, freeze-dried, and allowed to equilibrate with room moisture prior to analysis. The moisture contents of the experimental diets and freeze-dried excreta were determined in duplicate by drying at 135°C for 3 h. Diet and excreta N were determined in duplicate using the microKjeldahl method on a Kjeltech 1028 distilling unit (US Tecator Inc., Herndon, PA). The gross energy (**GE**) contents of the experimental diets and the excreta were determined in duplicate using an adiabatic bomb calorimeter (model 1281, Parr Instrument Company, Moline, IL). In addition, the GE contents of the crude glycerol and of chemically pure (≥99%) glycerol (catalog number G5516; Sigma-Aldrich, St. Louis, MO) were determined in triplicate. The contents of acid-insoluble ash in the experimental diets and the excreta were analyzed in triplicate according to procedures by Vogtmann et al. (1975).

The AME_n content of the crude glycerol was estimated by a linear regression equation relating the experimental-diet AME_n values to the proportion of crude glycerol in each diet (Leeson and Summers, 2001; Sell et al., 2001). The contribution of AME_n from glucose·H_2O in all diets, calculated from the glucose percentage inclusion rate and the glucose AME_n value (3,640 kcal/kg), was subtracted from glucose-containing diets (Hill and Anderson, 1958; Sell et al., 2001). The AME_n value of the experimental diet was calculated according to the method listed by Leeson and Summers (2001) as

Table 1. Composition of the basal diet (as-is basis)

Item	Amount, %
Ingredient	
Corn	39.82
Soybean meal (48% CP)	21.00
Glucose·H_2O	15.00
Meat and bone meal (50% CP)	9.60
Calcium carbonate[1]	9.23
Vegetable oil	3.25
Celite	1.00
Vitamin premix[2]	0.35
Trace mineral premix[3]	0.30
DL-Met	0.27
L-Thr	0.08
Sodium chloride (iodized)	0.10
Total	100.00
Calculated composition	
CP	17.85
AME_n, kcal/kg	2,875
Ether extract	5.87
Linoleic acid	2.16
Ca	4.51
P (nonphytate)	0.51
K	0.69
Na	0.21
Cl	0.29
Met	0.52
Met + Cys	0.77
Lys	0.96

[1]Supplied as a 50:50 mix of fine (0.14-mm mean diameter) and coarse (2.27-mm mean diameter) particles.

[2]Supplied per kilogram of diet: vitamin A, 9,259 IU; vitamin D_3, 3,086 ICU; vitamin E, 15 IU; vitamin B_{12}, 12 μg; riboflavin, 6 mg; niacin, 31 mg; D-pantothenic acid, 11 mg; choline, 386 mg; vitamin K, 2 mg; folic acid, 0.5 mg; vitamin B_6, 2 mg; thiamine, 2 mg; D-biotin, 0.05 mg.

[3]Supplied per kilogram of diet: manganese, 70 mg; zinc, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

Table 2. Characterization of the crude glycerol[1] fed to laying hens (as-is basis)

Characteristic	Amount	Method
Glycerol, %	86.95	ASTM D 6584-00E1[2,3]
Moisture, %	9.22	AOAC 984.20[4,5]
Methanol, %	0.028	Gas chromatography (proprietary method)[2]
CP, %	0.41	AOAC 990.03[4,5]
Crude fat, %	0.12	AOAC 920.39 (A)[4,5]
Ash, %	3.19	AOAC 942.05[4,5]
Na, %	1.26	AOAC 956.01[4,5]
Cl, %	1.86	AOAC 9.15.01, 943.01[4,5]
K, %	<0.005	AOAC 956.01[4,5]
Color, Fat Analysis Committee color standard	<1	AOCS Cc13a-43[4,6]
pH	5.33	Not reported[2]

[1]Supplied by Ag Processing Inc., Sergeant Bluff, IA; lot number GB605-03. The principal feedstock at this facility is soybean oil (Glycine max).

[2]Values reported by Ag Processing Inc.

[3]American Society for Testing and Materials (2006).

[4]Analyzed by the experiment Station Chemical Laboratories, University of Missouri, Columbia, MO.

[5]Association of Official Analytical Chemists (1990).

[6]American Oil Chemists' Society (2000).

$$AME_n = GE_{Diet} - \frac{GE_{Excreta} \times AIA_{Diet}}{AIA_{Excreta}} - 8.22 \times N_{Retained}$$

where AME_n (kcal/kg) = N-corrected apparent metabolizable energy content of the diet; GE_{Diet} and $GE_{Excreta}$ (kcal/kg) = GE of the diet and excreta, respectively; AIA_{Diet} and $AIA_{Excreta}$ (%) = acid insoluble ash in the diet and excreta, respectively; 8.22 (kcal/kg) = energy value of uric acid; and $N_{Retained}$ (g/kg) is the N retained by the hens per kilogram of diet consumed. The retained N was calculated as

$$N_{Retained} = N_{Diet} - \frac{N_{Excreta} \times AIA_{Diet}}{AIA_{Excreta}}$$

where N_{Diet} and $N_{Excreta}$ (%) = N contents of the diet and excreta, respectively.

The experimental design was a completely randomized design with 4 dietary treatments and 6 replications per treatment (Morris, 1999). The cage containing 2 hens was the experimental unit. The AME_n value of the crude glycerol was estimated as the slope of the linear relationship between the inclusion rate of dietary crude glycerol (independent variable) and the glucose-corrected AME_n value of the experimental diet (dependent variable) using JMP 6.0.3 (SAS Institute Inc., Cary, NC). The effects of dietary crude glycerol on egg production, egg weight, egg mass, and feed consumption were analyzed by ANOVA using JMP. The ANOVA model included only the effects of dietary crude glycerol content, and treatment means were separated using linear, quadratic, and cubic orthogonal polynomial contrasts (Morris, 1999). The GE value of crude glycerol used in the study was compared with its AME_n value using a 2-tailed *t*-test with n = 3 for GE values and n = 24 for AME_n values (Snedecor, 1946). Probability values less than or equal to 0.05 were considered significant. Where appropriate, means and associated SEM are reported in the text on an as-is basis.

RESULTS

The mean BW of the hens was 1.37 ± 0.01 kg (n = 24) at the start of the experiment with no significant difference among treatments ($P = 0.60$). The increases in dietary AME_n values attributed to substitution of crude glycerol for glucose·H_2O increased linearly with increasing crude glycerol content ($P < 0.001$); there were no quadratic or cubic effects ($P > 0.1$). The AME_n value of the crude glycerol tested was 3,805 ± 238 kcal/kg (Figure 1) and was not different ($P > 0.1$) from its GE value (3,625 ± 26 kcal/kg) as revealed by a 2-tailed *t*-test. Feed consumption (104 ± 4 g/d), egg production (93.0 ± 2.6%), egg weight (56.1 ± 0.9 g), or egg mass (52.2 ± 1.9 g/d) were not affected ($P > 0.1$) by the dietary treatments in the 10-d experiment. The GE value of chemically pure (≥99%) glycerol was 4,305 ± 30 kcal/kg.

DISCUSSION

The GE content of the crude glycerol used in the present experiment (3,625 kcal/kg) was similar to the 3,596 kcal/kg reported by Cerrate et al. (2006), who calculated the ME content as 98% of the analyzed GE content. In the present experiment, the AME_n value of crude glycerol was found to be 3,805 kcal/kg and was not different from its GE value. Rosebrough et al. (1980) used a ME value of 4,200 kcal/kg for glycerol in a turkey hen experiment,

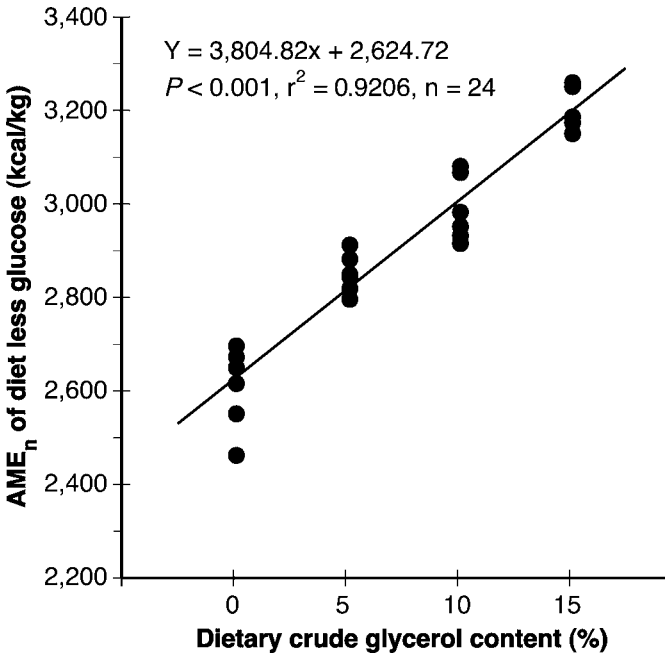

Figure 1. Relationship between the dietary inclusion rate of crude glycerol and the glucose-corrected AME_n value of the experimental diets for laying hens (each dot represents data from 1 cage containing 2 hens).

but did not specify the chemical purity or origin of the glycerol used. In comparison, the NRC (1994) lists a ME_n content of corn grain of 3,350 kcal/kg and that of crude soybean oil between 8,020 to 8,650 kcal/kg. Hence, crude glycerol is estimated to contain about 14% more ME_n than that of corn grain, and less than half of that contained in crude soybean oil. Moreover, crude glycerol supplies only energy and not nutrients (e.g., amino acids, phosphorus, and essential fatty acids) as do corn grain and soybean oil, which should be considered when assigning a monetary value to crude glycerol. The chemically pure sample of glycerol contained 4,305 kcal/kg of GE, suggesting that the AME_n content of crude glycerol may be a direct function of its glycerol content.

The crude glycerol tested in the present experiment had a relatively high content of Na and—because the Na content was not equalized among the treatment diets—the excreta from hens fed the 15% crude glycerol diet was considerably wetter than that from other treatments. Moreover, crude glycerol is a viscous liquid, and flow characteristics of diets containing 10 and 15% crude glycerol were noticeably poorer than that of the control diet, an effect also observed by Kijora et al. (1995) and Cerrate et al. (2006). Based on growth performance, Simon et al. (1996), Kijora et al. (1995), and Cerrate et al. (2006) recommended feeding glycerol at 5 to 10% of the diet for broiler chickens and pigs, respectively, which is consistent with the observations of physical effects on the feed from the present experiment. The results of this study show that the energy in crude glycerol is used efficiently by laying hens and has an AME_n content of 3,805 kcal/kg (as-is basis), 14% higher than that of corn grain.

ACKNOWLEDGMENTS

This project was supported by the Hatch Act, State of Iowa funds, and USDA-ARS. The authors acknowledge the in-kind donation of the crude glycerol (Ag Processing Inc., Sergeant Bluff, IA), laying hens (Southwest Iowa Eggs, Massena, IA), vegetable oil (Feed Energy Company, Des Moines, IA), calcium carbonate (ILC Resources, Des Moines, IA), vitamins (DSM Nutrition, Ames, IA), and meat and bone meal (Darling International, Des Moines, IA) used in this study. Moreover, the assistance provided by personnel in the Bregendahl and Kerr laboratories and at the Iowa State University Poultry Science Research Center is greatly appreciated.

REFERENCES

American Oil Chemists' Society. 2000. Official methods and recommended practices of the AOCS. 5th ed. Am. Oil Chem. Soc., Champaign, IL.

American Society for Testing and Materials. 2006. Annual book of American Society for Testing and Materials Standards International, Volume 05.04, Petroleum products and lubricants (IV): D6557—Latest. ATSM Int., West Conchohocken, PA.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.

Brisson, D., M.-C. Vohl, J. St-Pierre, T. J. Hudson, and D. Gaudet. 2001. Glycerol: A neglected variable in metabolic processes? Bioessays 23:534–542.

Cerrate, S., F. Yan, Z. Wang, C. Coto, P. Sacakli, and P. W. Waldroup. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. Int. J. Poult. Sci. 5:1001–1007.

Hill, F. W., and L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603.

Kijora, C., H. Bergner, R.-D. Kupsch, and L. Hagemann. 1995. Glycerol as a feed component in diets of fattening pigs. Arch. Anim. Nutr. 47:345–360.

Leeson, S., and J. Summers. 2001. Nutrition of the chicken. 4th ed. Univ. Books, Guelph, Ontario, Canada.

Lin, E. C. C. 1977. Glycerol utilization and its regulation in mammals. Annu. Rev. Biochem. 46:765–795.

Ma, F., and M. A. Hanna. 1999. Biodiesel production: A review. Bioresour. Technol. 70:1–15.

Morris, T. R. 1999. Experimental design and analysis in animal sciences. CABI Publ., Oxon, UK.

National Biodiesel Board. 2007. Estimated US biodiesel sales. http://www.biodiesel.org/pdf_files/fuelfactsheets/Biodiesel_Sales _Graph.pdf Accessed May 23, 2007.

NRC. 1994. Nutrient requirements of poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Rosebrough, R. W., E. Geis, P. James, H. Ota, and J. Whitehead. 1980. Effects of dietary energy substitutions on reproductive performance, feed efficiency, and lipogenic enzyme activity on large white turkey hens. Poult. Sci. 59:1485–1492.

Sell, J. L., S. Jin, and M. Jeffrey. 2001. Metabolizable energy value of conjugated linoleic acid for broiler chicks and laying hens. Poult. Sci. 80:209–214.

Simon, A., H. Bergener, and M. Schwabe. 1996. Glycerol—feed ingredient for broiler chickens. Arch. Anim. Nutr. 49:103–112.

Snedecor, G. W. 1946. Statistical Methods. 4th ed. Iowa State Coll. Press, Ames, IA.

Tao, R. C., R. E. Kelley, N. N. Yoshimura, and F. Benjamin. 1983. Glycerol: Its metabolism and use as an intravenous energy source. J. Parenter. Enteral Nutr. 7:479–488.

Thompson, J. C., and B. B. He. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. Appl. Eng. Agric. 22:261–265.

van Gerpen, J. 2005. Biodiesel processing and production. Fuel Process. Technol. 86:1097–1107.

Vogtmann, H., H. P. Pfirter, and A. L. Prabucki. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br. J. Poult. Sci. 16:531–534.